The Examiner has rejected claims 2-8, 10-11, 13-22 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Bok WO in view of Wang, et al., "Effect of *Drynaria Baronii* Rhizome Extracts on the Proliferation in Osteoblast-like UMR106 Cells," Pharmaceutical Biology, 2001, Vol. 39, No. 4, pp. 259-262 ("Wang").

The Examiner asserts that Bok WO teaches pharmaceutical compositions which comprise hesperidin or hesperitin for inhibiting the HMG-CoA reductase activity in mammals, and that hesperidin and hesperitin can be extracted from citrus peels or synthesized, and can be incorporated into food and beverages. The Examiner acknowledges that Bok does not teach the use of hesperidin in a method for stimulating bone formation and/or inhibiting bone resorption. Thus, the Examiner writes:

> "It would have been obvious to one of ordinary skill in the art at the time of the invention to have employed hesperidin isolated from citrus and incorporated into food and drink, as taught by [Bok WO], and employed this active component as a treatment agent for bone disorders, namely osteoporosis. One would be motivated to employ hesperidin for such therapeutic applications because based on the teachings of Wang, hesperidin, the active constituent in *Drynaria baronii* fractions was found to have direct stimulating effects on the proliferation of osteoblast-like cells. Therefore, since the active ingredient is hesperidin, it would be expected that no matter the source of isolation, the same mode of action would be expected to be observed. Thus, one would expect, with a reasonable degree of success, that hesperidin may be employed as a method of stimulating bone formation and in turn treating a number of disorders in which bone remodeling is critical."

(Office Action, pp. 4-5). With all due respect, Applicants submit that the rejection is based purely on hindsight reasoning and, as such, must be reconsidered and withdrawn.

As discussed in the last Response, Bok teaches that HMC-CoA reductase is involved in hyperlidemia, artheriosclerosis, angina pectoris, stroke and hepatic diseases. Such diseases are clearly different from osteoporosis and other bone-related diseases. Nothing in Bok WO would lead one of skill in the art to reasonably expect that

a compound which inhibits HMG-CoA reductase could have an effect on bone resorption and/or bone formation.

Wang is directed to the biological activity of *Drynaria baronnii* rhizome extracts. Wang teaches that ethyl acetate fraction and n-butanol fraction of *Drinaria baronnii* rhizome at a concentration of 0.4 mg/ml stimulate the proliferation of UMR106 cell so-called osteoblast-like cells. As the Examiner certainly knows, organic extracts are generally complex mixtures comprising a multitude of compounds. Wang does not provide any chemical analysis of the fractions obtained from *Drinaria baronnii* rhizome. Thus, based simply on the experimental data provided by Wang, it is impossible to determine the chemical composition of the said organic fractions (i.e. whether or not hesperidin is present). In this regard, Wang clearly states that *"Up to now it is not clear what are the effective constituents of these medicines, including Drynaria baronii" (p. 262).* Wang emphasizes that *"no constituent isolation has been reported for Drynaria baronii."*

In discussing the state of the art, Wang mentions that Zhou *"determined two flavonoids, naringenin and hesperedin, in Drynaria baronii."* However, Wang does not discuss the analytic methods used by Zhou and, even more importantly, without providing any teaching or suggestion that hesperidin – as opposed to naringenin or another, unidentified component – is linked to the biological activity of *Drynaria baronii* extracts on cell proliferation described in the Wang article. Wang concludes by stating that *"it may be possible to isolate the active constituents from the rhizome of Drynaria baronii though activity-guided fractionation".* Thus, Wang clearly states that the active constituents of *Drynaria baronii* were unknown to Wang.

Based on the foregoing, it is apparent that, in the absence of Applicants'

own disclosure, Wang neither discloses nor suggests that (a) hesperidin is present in either the ethyl acetate or butanol fractions from *Drynaria baronii* extracts, and/or (b) hesperidin is the active constituent responsible of cell proliferation. Thus, in considering the teaching of Wang as a whole, a person of skill in the art would have understood that knowledge of the composition of the *Drynaria baronii* extract was very poor, making it impossible to have reasonably predicted or known what compounds from the extracts were responsible of the cell proliferation induction. Applicants respectfully submit that the Examiner has used hindsight in asserting that Wang teaches that hesperidin is the active compound in the *Drynaria baronii* extracts responsible for cell proliferation

The teachings of Bok WO do not cure the deficiencies of Wang since, as noted above, Bok WO does not discuss or relate to bone resorption or bone stimulation.

For these reasons alone, the rejection over Bok WO in view of Wang should be withdrawn.

Moreover, a careful review of Wang's disclosure shows that its conclusions are scientifically suspect. First, Wang uses UMR106 cells for performing the cell proliferation assay. Such a cell line is not a primary osteoblast cell line but an osteocarcoma one (i.e. cancer cell line), which necessarily displays a metabolism distinct from that of normal cells. Thus, the physiological relevance of these results is questionable because of physiological divergences between osteoblasts and osteocarcomas which mean that one cannot reliably extrapolate results obtained for UMR106 cells to primary osteoblast cell lines.

In addition, when considering the results shown in Wang's Table 2, it is not clear that ethyl acetate and n-butanol fractions actually enhance cell proliferation. The

asserted stimulation of cell proliferation is observed only at a concentration of 0.4 mg/ml. At lower or higher concentrations, no clear effect is observed. Since Wang does not provide a comprehensive dose-effect study, the one skilled in the art cannot reasonably determine if the effect observed at 0.4 mg/ml for n-butanol and ethyl acetate fractions is an artifact or not. In other words, one of skill in the art cannot make reasonable conclusions, based on the data presented in Wang, of the biological effect of the n-butanol and ethyl acetate fractions on UMR106 proliferation. In this respect, the results obtained for the aqueous fraction are also unsettling: the absorbance for this fraction, which is clearly claimed as inactive, varies from 0.134 to 0.156. This further illustrates the uncertainty of the assay performed by Wang.

Finally, Wang suggests determining the active constituents from *Drynaria baronii* rhizome fractions though activity-guided fractionation. The activity assay proposed by Wang is a cell proliferation assay on UMR106 cell line. However, in the present application, the Applicants showed that hesperitin enhances the differentiation of hPOS-tert cells (which are immortalized osteoblasts) in a dose-dependent manner (see Example 2). Such a result was confirmed by the recent study of Trzeciakiewicz (attached as Exhibit A, see abstracts and paragraph entitled "effect of Hp7G on osteoblast proliferation and
differenciation" on p.673) which further describes that hesperitin and its metabolite (hesperetin7- 0-glucuronide) did not affect the proliferation of primary rat osteoblasts.

In other words, hesperetin and its derivatives are unable to induce cell proliferation.

Thus, following the disclosures of Wang, a person of skill in the art seeking

to determine the active constituents from the *Drynaria baronii* extracts would have (i) performed fractionations and (ii) assessed the biological activity of the resulting fractions by the *in vitro* assay as described in Wang so as to identify a compound able to induce the proliferation of osteoblast cell lines. However, using such a methodology, the person of skill in the art could not have isolated hesperetin or a derivative thereof as the active compound since hesperetin and its derivatives are unable to enhance osteoblast proliferation.

Therefore, since Wang teaches that the enhancement of cell proliferation is the differential criteria for identifying active compounds from *Drynaria baronii* extracts, Wang, even combined with the teachings of Bok WO, would not lead the one of skill in the art to the present invention because hesperetin and its derivatives have no effect on oesteoblast proliferation.

In view of the foregoing, Applicants respectfully submit that the presently claimed invention would not have been obvious over Bok WO in view of Wang, and request reconsideration and withdrawal of the rejection under § 103(a)

Conclusion

In view of the foregoing, this application is now in condition for allowance.

If deemed helpful, the Examiner is invited to contact the undersigned.

Respectfully submitted,

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